Designed Arginine Rich RNA-Binding Peptides with Picomolar Affinity

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Supplemental Information

RNA and Peptide Synthesis

Peptides were generated on an Applied Biosystems 432A peptide synthesizer using solid phase, F-Moc chemistry. Crude peptides were deprotected by TFA/ethanedithiol/thioanisole treatment and purified on a C-18 reverse phase HPLC column to a final purity greater than 95% (MALDI-TOF, Analytical C-18 HPLC). Peptides without a naturally occurring tryptophan or tyrosine residue were synthesized with a carboxy-terminal Gly-Tyr tag for quantification purposes.

Unlabeled RNA hairpins ($\lambda boxB_{R15}$ and $P22boxB_{L15}$) were synthesized by *in vitro* transcription using T7 RNA polymerase.[1] The RNA was purified by 20% urea-PAGE, desalted on a NAP column (Amersham Pharmacia), and freeze-dried. RNA was quantified by UV absorption at a wavelength of 260 nm.

Labeled RNA hairpins containing 2-amino purine (2AP) at loop position 2 (2AP-2), 3 (2AP-3), or 4 (2AP-4) were constructed by automated RNA synthesis using either 2-aminopurine-TOM-CE phosphoramidite or 2'-O-methyl 2-aminopurine phosphoramidite (Glen Research, Sterling, VA).

Steady-State Fluorescence Measurements

Measurements were conducted following the procedures of Barrick et. al.[2] Titrations were performed on a Shimadzu Spectrofluorophotometer at 20º C with Excitation/Emission wavelengths at 310/370 nm . Peptides were titrated iteratively into a constantly stirred solution of 2AP labeled RNA hairpin (20-200 nM RNA). Binding buffer contained 20 mM Tris-OAc, with a variable concentration of KOAc (15 mM-500 mM) at pH 7.5. Binding Constants were calculated for a one step binding mechanism by nonlinear least squares regression using the computer program DynaFit.[3] All isotherms were fit with < 10% uncertainty. Relative 2AP fluorescence change (F) is reported as a fraction; $F = (F_F/F_O)$ where F_O and F_F are the initial and final fluorescence respectively. Binding constants required a change in fluorescence of greater than 10% for reliable fitting. Salt dependence values $(-\delta \log K_{obs})(\delta \log[M^+])^{-1}$ were determined from five or more binding constants within a range of salt concentrations (50-500mM KOAc).

Stopped-Flow Fluorescence Measurements

Experiments were conducted following the procedures of Lacourciere et al.[4] Measurements were performed at 20º C under standard buffer conditions (20 mM Tris-OAc, 50 mM KOAc, pH 7.5) using a stop-flow device from Applied Photophysics (Surray, U.K.) in two-syringe mode. Fluorescence excitation was performed at 310 nm and emmision was measured with a filter cutoff > 360 nm. The

association rate (k_{on}) was determined for the λ_{N22}-λboxB complex to be 7 x 10^8 $s^{-1}M^{-1}$. A similar rate was observed for the $P22_{N21}$-P22boxB complex. Dissociation rate values (k_{off}) were determined for each interaction by infusing labeled complex with both 100x and 500x concentration of unlabeled competitor boxB at time zero.

Circular Dichroism Spectroscopy

Spectra were recorded on an Aviv 62 DS CD spectrofluorimeter in 10 mM potassium phosphate buffer at 20º C. Spectra for the peptide-RNA complexes were determined by subtracting spectra with the equivalent concentrations of RNA and of peptide from the spectra of the complex. Helix content was calculated from ellipticity at 222 nm (θ_{222}), using –40,000(1-2.5/n) and 0 deg cm^2 $dmol^{-1}$ as values for $\theta_{helix222}$ and $\theta_{coil222}$ respectively (n is the number of amino acids in the peptide).[5] Helicity = $(\theta_{observed})/(\theta_{helix} + \theta_{aromatic})$. The contribution of Trp was assumed to be –2300 ± 600 ($\theta_{aromatic}$) while the GY tag of the $P22_{N21}$was assumed to contribute insignificant helicity and aromaticity to the CD spectrum.[6]

NMR Spectroscopy

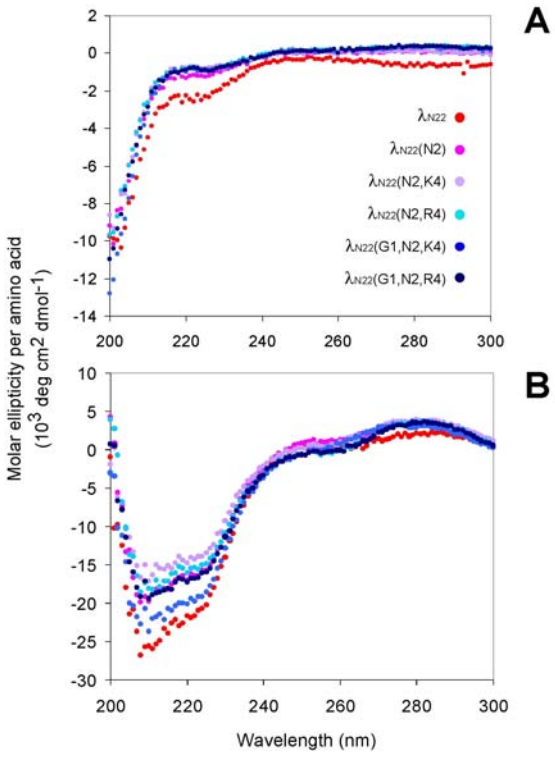
Samples were prepared in NMR buffer: 50 mM NaCl, 10 mM phosphate, pH 6 in H_2O:D_2O (90:10, vol:vol) as previously described.[2] NMR spectra were collected at 25ºC on a Varian INOVA 600-MHz spectrometer. A modified double gradient echo Watergate solvent-suppression pulse sequence was used to suppress the solvent peak.[7] Assignments were based on reported work.[8]

Supplemental Table 1. [a]

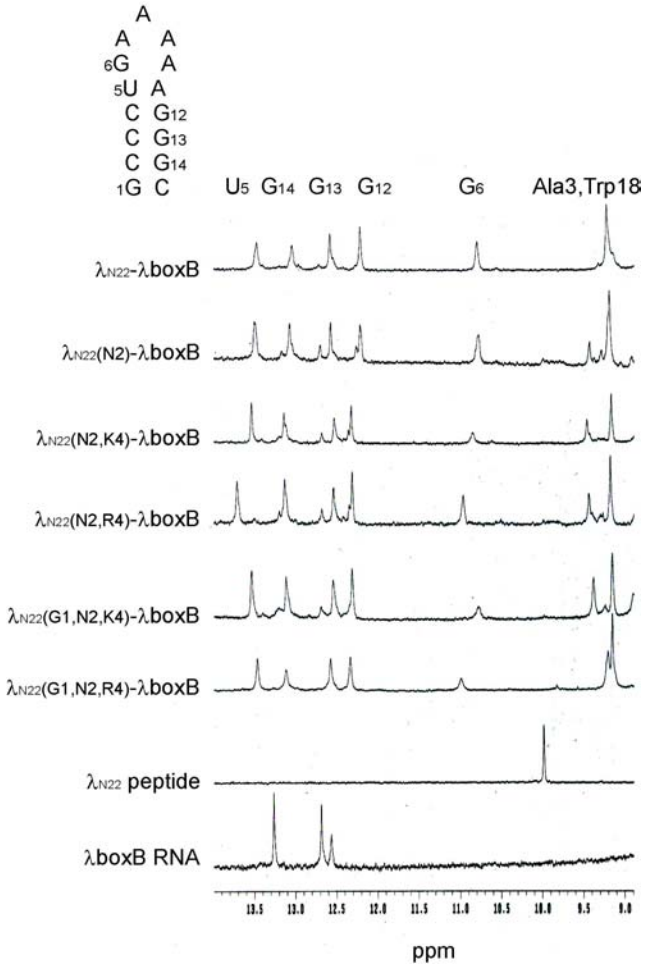
	P22boxB RNA		λboxB RNA				
					Fluorescence (F)		
peptides	$K_{d(70)}$ (pM)	k_{off}/k_{on} (pM)	$K_{d(70)}$ (pM)	k_{off}/k_{on} (pM)	2AP-2	2AP-3	2AP-4
$P22_{N21}$	5.0 ± 1.4	12	850 ± 290		1.60	0.53	1.00
λ_{N22}			1,900 ± 690	1,000	0.32	0.22	2.35
λ_{N22}(N2)			170 ± 18	170	0.36	0.15	2.60
λ_{N22}(N2,K4)			97 ± 22	69	0.33	0.21	2.45
λ_{N22}(N2,R4)			23 ± 4.9	17	0.35	0.14	2.50
λ_{N22}(G1,N2,K4)			34 ± 7.9	49	0.35	0.12	2.42
λ_{N22}(G1,N2,R4)			12 ± 3.2		0.32	0.13	2.39

[a] Dissociation constants (K_d's) were determined from salt dependence measurements for 2AP-2 labeled complexes at standard salt conditions (70 mM [M^+]). Salt dependence values $\delta/\delta = (\delta \log K_{obs})(\delta \log[M^+])^{-1}$ were determined from five or more measurements over a range of 50-500 mM KOAc. Individual measurements were fit with < 10% error. Kinetic approximations of dissociation constants, $K_d = k_{off}/k_{on}$, were calculated using a rate of association value, k_{on} = 7 x 10^8 $s^{-1}M^{-1}$; rate of dissociation values were fit with < 10% error.

Supplemental Figure 1. Circular dichroism measurements for λ_{N22} and reciprocal mutants in free solution (**A**) and in complex with λboxB (**B**). Measurements were taken in 10mM phosphate buffer at 20°C. Concentration of peptide in free solution was 10μM. Peptide-RNA complexes were prepared at 6:5μM stoichiometry.



Supplemental Figure 2. NMR imino-spectra of λ_{N22}-λboxB native and reciprocal complexes. The Trp18 imino-peak shifts from 10.0 ppm in the free peptides to 9.2 ppm in the λboxB comlex. Samples were prepared in 50 mM NaCl, 10 mM phosphate buffer, pH 6 and titrated to 1:1 stoichiometry at 25°C. Assignments are based on reported work.[8]



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